Differential Susceptibility to Glyphosate among the *Conyza* Weed Species in Spain

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Greenhouse and laboratory experiments were conducted to investigate differences in glyphosate susceptibility among three species of the genus *Conyza* introduced as weeds in Spain: tall fleabane (*Conyza sumatrensis*), hairy fleabane (*Conyza bonariensis*), and horseweed (*Conyza canadensis*). Plant material was obtained from seeds collected in weed populations growing in olive groves and citrus orchards in southern Spain, with no previous history of glyphosate application. Dose−response curves displayed ED_{50} values of 2.9, 15.7, and 34.9 g ai ha^{-1}, respectively, for *C. sumatrensis*, *C. bonariensis*, and *C. canadensis* plants at the rosette stage (6−8 leaves). Significant differences were found among the three species in the glyphosate retention on leaves as well as the leaf contact angle. The species order according to glyphosate retention was *C. sumatrensis* > *C. bonariensis* > *C. canadensis*, while the mean contact angles of glyphosate droplets were 59.2, 65.5, and 72.9°, respectively. There were no significant differences among species in the absorption of [^{14}C]glyphosate (ranged from 37.4% for *C. canadensis* to 52.4% for *C. sumatrensis*), but the order among species was the same as glyphosate retention. The amount of radioactivity translocated from treated leaves was lower in *C. canadensis* as compared to the other two species (*C. sumatrensis* > *C. bonariensis* > *C. canadensis*). Combined, all of the studied parameters identified differential susceptibility to glyphosate among the *Conyza* species. Each species accumulated shikimate in leaf tissues following application of glyphosate at 200 g ai ha^{-1}. However, *C. canadensis* exhibited lower shikimate levels than the other two species at 168 h after herbicide application. For hairy fleabane, a greenhouse study explored its susceptibility to glyphosate at three developmental stages: rosette, bolting (stem height, 10−15 cm), and flowering. The ED_{50} was lower at the rosette stage (15.7 g ai ha^{-1}) as compared to bolting (86.6 g ai ha^{-1}), with the highest ED_{50} values occurring at flowering (117.5 g ai ha^{-1}); plants at the earlier developmental stage retained more glyphosate. These results agree with field observations that plants at early developmental stages are more sensitive to glyphosate.

KEYWORDS: *Conyza* spp.; glyphosate; susceptibility; laboratory and field assays

INTRODUCTION

Different species of the genus *Conyza* Less. (Asteraceae) are found in Europe as neophytes introduced from both North and South America. These invasive species, now considered to be among the most common plant species in the recipient territory (*1*), behave primarily as ruderal plants, inhabiting road margins, recently abandoned fields, riverbanks, urban wasteland, etc. *Conyza* spp. occur as weeds in more than 40 crops in 70 countries (*2*). Recently, *Conyza* spp. have increased in severity in fruit orchards and olive groves in southern Spain (*3*). Prior to conservation tillage practices in orchard (mainly citrus and olive trees) production, *Conyza* spp. were easily controlled with tillage (*4*). Even with the suppression of primary tillage, glyphosate application in early spring was an effective tool for *Conyza*

control. However, in a recent survey of consultants in southern Spain, *Conyza* spp., along with *Lolium* spp., were considered to be the most important weeds in fruit orchards despite extensive herbicide use (*5*). Several underlying factors could explain this, including application of glyphosate at later stages of weed development, which are more tolerant, differential susceptibility among *Conyza* species and the selection of biotypes that exhibit herbicide resistance (*6, 7*). In Spain, three annual *Conyza* species are widespread in citrus orchards and olive groves: hairy fleabane [*Conyza bonariensis* (L.) Cronq.], horseweed [*Conyza canadensis* (L.) Cronq.], and tall fleabane [*Conyza sumatrensis* (Retz.) E. Walker]. For all three species, seedling emergence is preferably in winter (*8*). Individual plants of *Conyza* spp. are prolific; *C. canadensis* can produce 50000−200000 seeds per

plant (*2*). Seeds are dispersed by the wind; distances up to 500 m have been measured for *C. canadensis* (*9*). The ability to disperse seeds a long distance suggests that effective management strategies of *Conyza* spp. should be practiced on larger spatial scales than the individual field (*9*). In addition, seed longevity in the soil has been documented up to 3 years in *Conyza* spp. in some crops (*10*). All of the above biological traits contribute to difficulty for the long-term management of *Conyza* spp. (*10*). For these reasons, preplant control is needed in orchards where conservation tillage is practiced. As an alternative, cover crops are being used to prevent or reduce intensive herbicide use in agriculture (*11*). However, dependence upon herbicides for controlling weeds persists, mostly due to their high efficacy for controlling the broad spectrum of weeds associated with each particular crop (*10, 12, 13*).

The herbicide glyphosate has been used extensively in agriculture worldwide for about 30 years, and today, it is the most commercialized herbicide in the world (*14*). Glyphosate belongs to the chemical group of amino acid synthesis inhibitors, and *N*-(phosphonomethyl) glycine is the chemical name (*15*). Following absorption, it is readily translocated with photosynthates from the leaves to meristematic tissue (*16*). In sensitive plants, glyphosate inhibits the enzyme 5-enolpyruvylshikimate phosphate synthase (EPSPS) (EC 2.5.1.19) (*17*). EPSPS is the critical and essential enzyme catalyzing the conversion of shikimate 3-phosphate and phosphoenolpyruvate to EPSPS (*18, 19*). The shikimic acid pathway plays a fundamental role in the biosynthesis of the aromatic essential amino acids phenylalanine, tyrosine, and tryptophan, as well as other important secondary compounds, such as auxins and allelochemicals (*20*).

In southern Spain, glyphosate has been recommended for *Conyza* spp. control in olive groves and citrus crops for many years. The objectives of this research were to (1) determine in greenhouse conditions the efficacy of glyphosate on *C. sumatrensis*, *C. bonariensis*, and *C. canadensis*; (2) characterize physical (foliar retention and contact angle) and physiological (shikimic acid accumulation, absorption and translocation of [^{14}C]glyphosate) factors that could explain differential sensitivity to glyphosate in *C. sumatrensis*, *C. bonariensis*, and *C. canadensis*; and (3) evaluate glyphosate efficacy in field conditions with *C. bonariensis* and *C. canadensis* at three growth stages.

MATERIALS AND METHODS

Herbicides. [^{14}C]Glyphosate (specific activity, 273.8 MBq mmol^{-1}; 95% radiochemical purity) was provided by Sigma Aldrich (France). A commercial formulation (Roundup Energy 45% w/v SL) of this herbicide was supplied by Monsanto Agricultura España S. L. and used for all in vivo and in vitro assays.

Plant Material and Growing Conditions. Glyphosate-susceptible populations of *C. sumatrensis*, *C. bonariensis*, and *C. canadensis* were used in the experiments described below. Seeds were collected in 2006 from orchards in Southern Spain. *C. bonariensis* and *C. canadensis* were collected from olive orchards in Córdoba, while *C. sumatrensis* was collected from citrus orchards in Huelva. In none of the cases had glyphosate ever been applied to control these weeds. The seeds were sown in 663 cm^3 pots filled with peat and were covered with transparent film until the seeds germinated. Seedlings were planted in pots (one plant per pot) containing a peat:sandy loam potting mixture (1:1 v/v) in a growth chamber at 28/18 °C (day/night), 16 h photoperiod, 850 μmol m^{-2} s^{-1} photosynthetic photon flux density, and 80% relative humidity.

Dose Response. Glyphosate was applied to plants at the rosette stage, that is, growth stage 14−15 according to the BBCH phenological scale (*21*), using a laboratory spray chamber at a height of 50 cm above the plants. Herbicide solutions were applied with flat fan nozzles (Tee Jet 8002 EVS) at 200 kPa and an output volume equivalent to 200 L ha^{-1}. Applied were the following doses: 0, 25, 50, 75, 100, 150, and 200 g ai ha^{-1}. In addition,

for *C. bonariensis*, a specie with more rapid vegetative growth as compared to the other two species, glyphosate was applied at two additional developmental stages: bolting (stems 10−15 cm in height; BBCH 32) and flowering (BBCH 55). The experiment was arranged in a completely randomized design with five replications per treatment (each replication with three plants). At 21 days after treatment, plants were cut at ground level, and the fresh weight was recorded and expressed as a percentage of untreated control plants. The same procedure was performed in the two additional growth stages of *C. bonariensis*. Herbicide rates inhibiting plant growth by 50% (ED$_{50}$) were determined for each species according to ref *22*. Data were pooled and fitted to a nonlinear, log−logistic regression model:

$$Y = c + \{(d - c)/[1 + (x/g)^b]\}$$

where *Y* is the fresh above-ground weight expressed as a percentage of the untreated control, *c* and *d* are coefficients corresponding to the lower and upper asymptotes, *b* is the slope of the line, *g* is the herbicide rate at the point of inflection halfway between the upper and the lower asymptotes and represents the ED$_{50}$, and *x* (independent variable) is the herbicide dose. Regression analysis was conducted using Sigma Plot 8.0 statistical software (*23*).

Foliar Retention. The methodology described by ref *24* was followed. Plants of each *Conyza* species at the rosette stage (BBCH 14−15) were sprayed with a colored glyphosate solution using the spray chamber as described above. In addition, the same treatment was applied to plants of *C. bonariensis* at the bolting (BBCH 32) and flowering (BBCH 55) stage. Treatment solutions contained glyphosate at 200 g ai ha^{-1} in a volume of 200 L and 100 mg L^{-1} Na-fluorescein. After the solution had dried on the foliage, plants were cut off at ground level and immersed for 30 s in 50 mL of 5 mM NaOH. Readings were made with a spectrofluorimeter at 490/510 nm. Plants were then placed at 80 °C for 72 h, and the dry matter was recorded. Four replications were used for each treatment (three plants per replication), and the experiment was repeated over time.

Contact Angle. The third leaf of each *Conyza* species at the rosette stage was cut off and placed onto a horizontal surface. Each leaf was treated with one, 1 μL droplet containing glyphosate corresponding to 200 g ai ha^{-1} in a volume of 200 L. Droplets were applied in the center of the adaxial surface. The pattern of droplet deposition was observed in a horizontal microscope (Leica MZ6 1,8X-4X). Images were captured with a camera [Leica Digilux 4.3 (1:2−8−4.5/8.3−24.9 mm) + Supermacro Leica Digimacro 4.3] adapted to one of the oculars of the microscope. The droplets were applied every 18 s. The contact angle was obtained by digital image analysis using the ImageJ program (*25*). Thirty replicates were measured for each *Conyza* spp.

Shikimic Acid Accumulation. Plants from each *Conyza* spp. at the rosette stage were treated with glyphosate (200 g ai ha^{-1} applied in a volume of 200 L) using a laboratory spray chamber as described above. Fifty milligrams of treated and untreated plant tissue was harvested simultaneously and frozen in liquid nitrogen at 24, 48, 72, 96, and 168 h after treatment, using the methodology described by ref *26*. Shikimic acid accumulation was optically determined using a Beckman DU-640 spectrophotometer at 380 nm, and results were expressed in μg per g fresh weight. The experiment consisted of eight treated and three nontreated plants per species with three replicates.

Absorption and Translocation of [^{14}C]Glyphosate. [^{14}C]Glyphosate was mixed with commercially formulated glyphosate to prepare a solution with a specific activity of 0.417 kBq μL^{-1} (both absorption and translocation experiments) and a glyphosate concentration of 1 g ai L^{-1} (200 g ai ha^{-1} in 200 L). The radiolabeled herbicide was applied in two droplets of 0.5 μL on the adaxial surface of the third leaf in each plant using a micropipet (LabMate + HTL). A total of 0.834 kBq was applied per plant. Preliminary assays showed that 96 h after herbicide treatment is the most suitable time for harvest to determine maximum glyphosate absorption and translocation and early visible symptoms of glyphosate effects. Therefore, treated plants were carefully removed from potting mix 96 h after herbicide treatment. The treated leaf was excised, and the unabsorbed [^{14}C]glyphosate was removed with 3 mL of a water−acetone (1:1 v/v) solution. Plants were separated into treated leaf, rest of the shoot, and root. The rinsate was mixed with 7 mL of scintillation liquid and analyzed by liquid scintillation spectrometry (LSS) (Scintillation Counter, Beckman

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Table 1. Parameters of the Model[a] Used To Calculate the Glyphosate Dose Required for 50% Plant Injury (ED$_{50}$) of *Conyza* spp. Plants at Rosette Stage (BBCH 14-15)

				ED$_{50}$		
	c	d	b	(g ai ha^{-1})	pseudo $r^{2\ b}$	P^c
C. sumatrensis	0.93	99.99	0.74	2.9 ± 3.1	0.98	<0.0001
C. bonariensis	0.84	99.99	2.09	15.7 ± 0.8	0.99	<0.0001
C. canadensis	1.14	100.20	5.20	34.9 ± 1.6	0.97	<0.0001

[a] $Y = c + \{(d - c)/[1 + (x/g)^b]\}$ where Y is the fresh weight (% of untreated control), x (independent variable) is the herbicide dose, c and d are the lower and the upper asymptotes, b is the slope of the line, and g (ED$_{50}$) is the effective dose required for 50% plant injury. Data were pooled and fitted to a nonlinear regression model. [b] Approximate coefficient of determination of nonlinear models with a defined intercept calculated as pseudo $r^2 = 1 - $ (sums of squares of the regression/corrected total sums of squares). [c] Probability level of significance of the nonlinear model. ED$_{50}$ mean values ± standard errors of the mean.

LS 6500). The plant tissue was dried at 60 °C over 72 h and combusted in a biological sample oxidizer, Packard Tri Carb 307. The $^{14}CO_2$ evolved was trapped and counted in 18 mL of a mixture of Carbo-Sorb E and Permafluor (9:9 v/v) (Perkin-Elmer, Packard Bioscience BV).

Radioactivity was quantified by LSS, and the percentage of herbicide absorbed was expressed as kBq in combusted tissue divided by kBq in combusted tissue + kBq in leaf washes and multiplied by 100. The experiment consisted of five replicates.

Phosphor Imaging. Visualization of herbicide translocation was performed using a phosphor imager (Cyclone, Perkin-Elmer, Packard Bioscience BV). Plants were treated with respective unlabeled and radiolabeled glyphosate as described for absorption and translocation experiments. Whole plants were gently rinsed, blotted dry, then pressed, and oven-dried (50 °C, 4 days); pressed plants were placed adjacent to 25 cm × 12.5 cm phosphor storage film during 6 h and scanned for radiolabel dispersion.

Field Experiments. In an olive grove near Cordoba in southern Spain ("Sotillo Bajo" farm), field experiments were carried out at three growth stages of *Conyza* plants: rosette (BBCH 14−15), bolting (BBCH 32), and flowering (BBCH 55). The olive grove was under super intensive management, with trees spaced 2 m between rows and 1.5 m apart within the row. At the time of herbicide application, the relative distribution of *C. canadensis* and *C. bonariensis* plants was 75 and 25%, respectively, while *C. sumatrensis* was absent. The experiment was conducted using a completely randomized design with three replications. The treated area for each treatment was 2 m wide by 5 m long. Treatments included an untreated control and glyphosate at 5 L ha^{-1} (2250 g ai ha^{-1}) (Roundup Energy 45% w/v SL) + Orosorb 0.8% (Alcohol etoxilate 8.92% w/v SL). The herbicide was applied at 2.3 km/h with a pneumatic backpack sprayer equipped with four flat fan nozzles (Albus API 11002) at 200 kPa and a height of 50 cm; the output volume was equivalent to 200 L ha^{-1}.

The herbicide effect was measured quantitatively on all treatments 90 days after application at the rosette stage (BBCH 14−15). At random, a 0.25 m^2 square was thrown down in each plot, and all of the plant material was cut off at ground level and placed into a paper bag (24.8 cm × 35.3 cm). Plant material was dried at 80 °C for 96 h, and the dry weight of each sample was recorded. Results were expressed in g of dry weight per m^2.

Statistical Analyses. Data were subjected to analysis of variance. Means were compared using Tukey's honestly significant difference (HSD) test at the 5% probability level. Statistical analyses were done using Statistix v 8.0 software.

RESULTS AND DISCUSSION

Dose Response. In greenhouse conditions, the three species of *Conyza* exhibited a high sensitivity to glyphosate at the rosette stage. However, differences were apparent among species. *C. sumatrensis* had an ED$_{50}$ of 2.9, followed by *C. bonariensis* with and ED$_{50}$ of 15.7 and *C. canadensis* with an ED$_{50}$ of 34.9 g ai ha^{-1}. These results show that *C. bonariensis* and *C. canadensis* are 5.4 and 12 times more tolerant, respectively, to glyphosate than *C. sumatrensis* (**Table 1**).

Table 2. Parameters of the Equation[a] Used To Calculate the Glyphosate Dose Required for 50% Plant Injury (ED$_{50}$) of *C. bonariensis* at Three Different Growth Stages

				ED$_{50}$		
growth stage	c	d	b	(g ai ha^{-1})	pseudo $r^{2\ b}$	P^c
rosette (BBCH 14−15)	0.84	99.99	2.09	15.7 ± 0.8	0.99	<0.0001
bolting (BBCH 32)	4.39	95.15	1.96	86.6 ± 24.8	0.98	<0.0001
flowering (BBCH 55)	9.67	95.25	2.41	117.5 ± 31.5	0.84	<0.0001

[a] Equation $Y = c + \{(d - c)/[1 + (x/g)^b]\}$, where Y is the fresh weight (% of untreated control), x (independent variable) is the herbicide dose, c and d are the lower and the upper asymptotes, b is the slope of the line, and g (ED$_{50}$) is the effective dose required for 50% plant injury. Data were pooled and fitted to a nonlinear regression model. [b] Approximate coefficient of determination of nonlinear models with a defined intercept calculated as pseudo $r^2 = 1 - $ (sums of squares of the regression/corrected total sums of squares). [c] Probability level of significance of the nonlinear model. ED$_{50}$ mean values ± standard errors of the mean.

Table 3. Spray Retention and Contact Angle of Glyphosate Solution for Three *Conyza* spp. Treated in the Rosette Stage

	μL spraying solution g^{-1} dry matter[a]	contact angle (deg)[a]
C. sumatrensis	779 ± 110 a	59.2 ± 1.1 c
C. bonariensis	599 ± 50 b	65.4 ± 0.9 b
C. canadensis	484 ± 80 c	72.9 ± 2.0 a

[a] Means within a column followed by the same letter are not significantly different at the 5% level as determined by the Tukey test. Mean values ± standard errors of the mean.

Infestations of *Conyza* spp. have become widespread in southern Spain. These species are problematic in no-tillage production systems, which rely extensively on glyphosate for weed control. Under field conditions, *Conyza* spp. are treated commonly at various stages of growth. Growth-dependent susceptibility to glyphosate could be a primary factor limiting treatment efficacy. In fact, our results indicate that, for *C. bonariensis*, the ED$_{50}$ value increased by 7.5-fold for plants treated at the rosette stage versus flowering (**Table 2**). The determination if reduced response to glyphosate was due to spray coverage or physiological changes in plant growth were not possible. Research by refs *27* and *28* reported that horseweed was consistently more sensitive to glyphosate in the rosette stage as compared to later developmental stages.

Foliar Retention and Contact Angle. The relationship of glyphosate retention and leaf contact angle was inverse when comparing the three species. *Conyza* spp. leaves retained a large amount of glyphosate solution, in the range of 500−800 μL glyphosate g^{-1} dry matter. However, spray retention of 1 g ai L^{-1} glyphosate aqueous solution was significantly greater for *C. sumatrensis* as compared to the other species (**Table 3**). In contrast, contact angles of droplets containing commercial glyphosate were significantly lower for *C. sumatrensis* than for the other species, in the range of 65−75° (**Table 3**). Research by ref *25* carried out on *Ambrosia artemisiifolia* shoots demonstrated that retention of glyphosate approached 800 μL g^{-1} dry matter, while contact angles of droplets containing commercial glyphosate were intermediate, in the 70−75° range. In comparison, retention values in difficult-to-wet species such as winter wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.) were lower than 50 μL g^{-1} (*29*).

Foliar retention was dependent upon the growth stage of the plants. Assays carried out on *C. bonariensis* showed that the spray retention rate of 1 g ai L^{-1} glyphosate aqueous solution was significantly higher ($P = 0.05$) at bolting (BBCH 32): 227 ± 20 μL g^{-1} as compared to the flowering (BBCH 55) with 184 ± 15 μL g^{-1}.
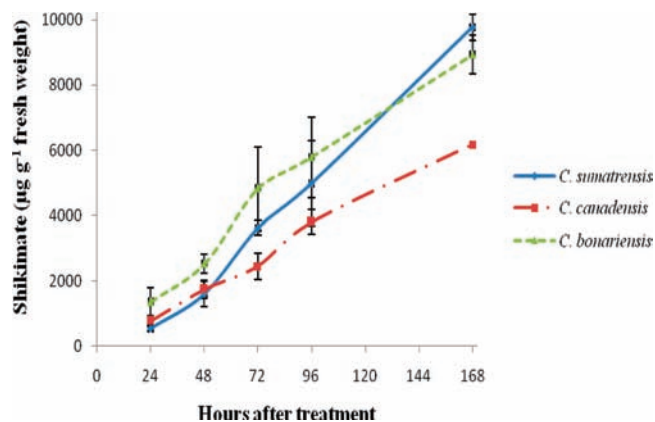
Figure 1. Shikimate accumulation of susceptible *Conyza* spp. plants following the application of glyphosate at 200 g ai ha^{-1}. Vertical bars represent $\pm$ standard errors of the mean.

Table 4. Absorption and Translocation of [^{14}C]Glyphosate in *Conyza* spp. at 96 h after Treatment

	% absorption[a]	translocation (% of absorbed)[a]		
		treated leaf	rest of plant	root
C. sumatrensis	52.4 ± 3.8 a	82.4 ± 1.6 b	5.9 ± 1.2 a	11.6 ± 1.5 a
C. bonariensis	48.8 ± 5.5 a	85.2 ± 0.6 b	6.4 ± 0.6 a	8.3 ± 0.1 a
C. canadensis	37.4 ± 9.0 a	93.0 ± 3.5 a	4.0 ± 2.8 b	2.8 ± 0.3 b

[a] Means within a column followed by the same letter are not significantly different at the 5% level as determined by the Tukey test. Mean values ± standard errors of the mean.

Figure 2. Phosphor images demonstrating movement of [^{14}C]glyphosate in representative *C. sumatrensis* (**a**), *C. bonariensis* (**b**), and *C. canadensis* (**c**) plants. Images were recorded 96 h after treatment; the intensity of red coloration indicates greater concentrations of glyphosate. The arrows point toward the initially treated leaf.

Spray retention and contact angle are important parameters of herbicide efficacy, because they determine the maximum amount of herbicide that can penetrate the target plant. In some cases, differences in spray retention and contact angle are thought to play a role in herbicide efficacy and selectivity (*30−32*).

Shikimic Acid Accumulation. Changes in shikimate levels in plants are specifically the result of inhibiting EPSPS (*33*), and have been used as a marker for EPSPS sensitivity in plants to glyphosate (*34*). Comparatively, a species with a lower accumulation of shikimate likely requires a higher level of glyphosate to be lethal (higher ED$_{50}$). Shikimate concentrations in the absence of glyphosate application were low and tended to be less than 1500 μg g^{-1} fresh weight. Following application, final shikimate concentrations ranged from 6000 to 10000 μg g^{-1} fresh weight under laboratory conditions (**Figure 1**). Shikimate concentrations increased in all *Conyza* spp. leaf tissues after glyphosate application, with amounts similar between *C. sumatrensis* and *C. bonariensis*. Nevertheless, at time points greater than 96 h, *C. canadensis* accumulated less shikimate than the other two species, which is consistent with the higher ED$_{50}$ for *C. canadensis* (**Table 1**); differences with respect to the other two species were significant (*P* = 0.05). Shikimate concentrations were in agreement with results for the foliar retention and contact angle experiments for the three *Conyza* species.

Absorption and Translocation of [^{14}C]Glyphosate. There were no significant differences in leaf absorption of applied [^{14}C]-glyphosate among the *Conyza* spp. (**Table 4**); the herbicide penetration was greater in *C. sumatrensis* than in *C. bonariensis* and *C. canadensis*. Clearly, these results, together with those found for herbicide retention, could explain the differential susceptibility among the three *Conyza* species. In addition, there was a clear difference among plant species in the amount of glyphosate translocated from the treated leaf to untreated leaves (rest of plant) and the roo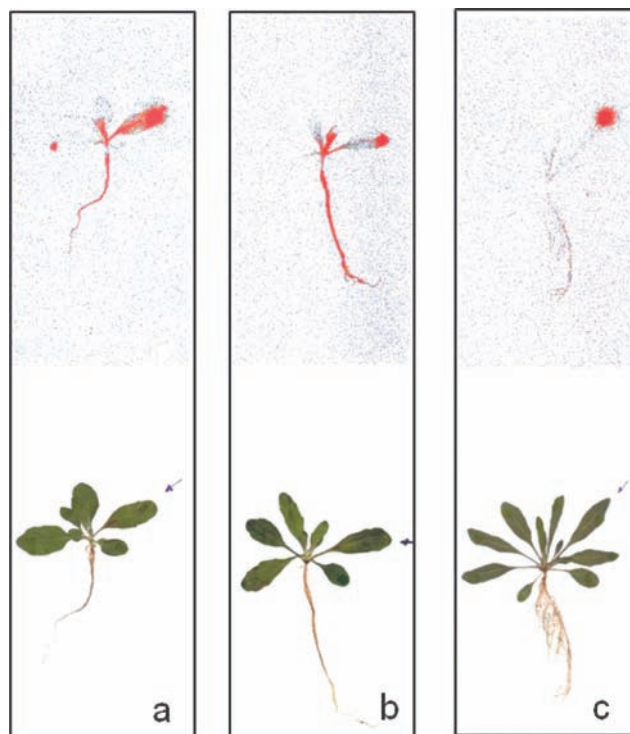t. Translocation of glyphosate from the treated leaf to roots was significantly different among *C. canadensis* (2.8%), *C. bonariensis* (8.3%), and *C. sumatrensis* (11.6%) (**Table 4**).

Phosphor Imaging. Differences in translocation of [^{14}C]glyphosate among the *Conyza* spp. plants were also visualized by phosphor imaging (**Figure 2**). In general, 96 h after herbicide application, translocation of glyphosate from the treated leaf was faster, and accumulation was greater in young leaves (leaf crown) as compared to old leaves in all *Conyza* spp. Translocation to roots was clearly different, with less glyphosate accumulation in *C. canadensis* (**Figure 2c**) as compared to *C. bonariensis* (**Figure 2b**) and *C. sumatrensis* (**Figure 2a**). In this experiment, glyphosate damage (leaf chlorosis) was evident 96 h after glyphosate treatment in the leaf crown of *C. sumatrensis* and *C. bonariensis*, while only the tissue at the point of glyphosate application in *C. canadensis* plants was chlorotic. This visual difference was consistent with the differences observed in glyphosate retention, absorption and translocation, as well as shikimate accumulation.

Field Experiments. All of the *Conyza* plants in this field study were susceptible to commercial doses of glyphosate (Roundup Energy 45% w/v SL). However, different levels of control were found depending upon the application time (**Table 5**). Biomass reductions of the mixed stand of *Conyza* spp. exceeded 85% for applications at the rosette and bolting stage as compared to untreated plants, but reductions were only 45.8% for plants treated during flowering (**Table 5**). It is understood that *C. canadensis* may not have responded similarly to glyphosate as *C. bonariensis*, but a visual inspection of the field site indicated that the distribution of each species was rather uniform. Differential sensitivity between *C. canadensis* and *C. bonariensis* may explain why the dominant species in this field site was *C. canadensis*.

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Table 5. Dry Weight of above Ground Biomass of *C. bonariensis* and *C. canadensis* at Different Growth Stages in an Olive Orchard in Córdoba, Spain[a]

growth stage	dry weight (g m^{-2})[b]	% reduction with respect to untreated plants[c]
rosette	23.8 ± 6.8	96.6
bolting	95.7 ± 16.9	86.5
flowering	385.7 ± 67.1	45.8

[a] Treatment: glyphosate 5 L ha^{-1} (2250 g ai ha^{-1}) + Orosorb 0.8%. [b] The herbicide effect was measured quantitatively at 90 days after treatment of the rosette stage (BBCH 14-15) plants. [c] Untreated plants: 711.9 (± 151.4) g dry weight m^2.

Results demonstrate that the three species of *Conyza* in southern Spain naturally differ in response to glyphosate. Although field trials determined that each species could be controlled with labeled application rates, large differences in response to glyphosate were observed among the species. Dose−response assays confirmed that *C. sumatrensis* and *C. bonariensis* are more susceptible to glyphosate than *C. canadensis*, with the ED$_{50}$ *C. canadensis*/ED$_{50}$ *C. sumatrensis* in BBCH 14−15 stage greater than 10-fold. This can be explained by the poor herbicide retention of this species (1.6 lesser than *C. sumatrensis*), as well as reduced leaf contact angle.

Once glyphosate reaches the leaf surface, physiological factors contributed to reduced movement of glyphosate to the target site. It was determined that 96 h after treatment, *C. canadensis* absorbed up to 15% less glyphosate and translocated up to 11% less glyphosate as compared with the other two *Conyza* species. A reduction in the amount of glyphosate available at the target site in different plant parts for *C. canadensis* versus the other *Conyza* spp. also likely contributed to reduced sensitivity of *C. canadensis*. This resulted in less glyphosate reaching the target site; *C. canadensis* exhibited shikimate levels up to 40% lower than *C. sumatrensis* at 168 h after treatment.

Within each species, sensitivity to glyphosate was reduced as plants matured from the rosette to early bolting to the flowering stage. Optimum management of *Conyza* spp. should include labeled application rates at the proper timing (rosette stage). Although growers may prefer to increase rates as the treated size of plants increases, this strategy may lead to greater selection pressure for resistance. As the use of glyphosate continues in olive groves in Spain, it is expected that a shift in species will occur, favoring *C. canadensis*. In the field trial where glyphosate was applied, the distribution of *Conyza* spp. already favors *C. canadensis*.

LITERATURE CITED

(1) Thébaud, C.; Abbott, R. J. Characterization of invasive *Conyza* species (Asteraceae) in Europe: Quantitative trait and isozyme analysis. *Am. J. Bot.* **1995**, *82*, 360–368.

(2) Holm, L.; Doll, J.; Holm, E.; Pancho, J.; Herberger, J. *World Weeds, Natural Histories and Distribution*; J. Wiley: New York, 1997; pp 226−235.

(3) Urbano, J. M.; Borrego, A.; Torres, V.; Leon, J. M.; Jimenez, C.; Dinelli, G.; Barnes, J. Glyphosate-resistant hairy fleabane (*Conyza bonariensis*) in Spain. *Weed Technol.* **2007**, *21*, 396–401.

(4) Brown, S. M.; Whitwell, T. Influence of tillage on horseweed (*Conyza canadensis*). *Weed Technol.* **1988**, *2*, 269–270.

(5) Calha, I. M.; Rocha, F.; Ruiz-Santaella, J. P.; Cruz-Hipolito, H. E. Two decades of herbicide resistance in the Iberian Peninsula. *J. Plant Dis. Prot.* **2008**, *21*, 79–84.

(6) González-Torralva, F.; Cruz-Hipolito, H. E.; Mülleder, N.; Domínguez-Valenzuela, J. A.; De Prado, R. *Resistance of Conyza canadensis to Glyphosate in Spain*. In XIIIth International Conference on Weed Biology, Dijon, France, AFPP: Dijon, Francia, 2009; pp 381−386.

(7) Heap, I. International Survey of Herbicide Resistant Weeds; www.weedscience.com (Accessed October, **2009**).

(8) Recasens, J.; Conesa, J. A. *Malas Hierbas en Plántula: Guía de Identificación*; Ediciones de la Universitat de Lleida: Lleida, Spain, 2009; pp 143−147.

(9) Dauer, J. T.; Mortensen, D. A.; VanGessel, M. J. Temporal and spatial dynamics of long-distance *Conyza canadensis* seed dispersal. *J. Appl. Ecol.* **2007**, *44*, 105–114.

(10) Wu, H.; Walker, S.; Rollin, M. J.; Tan, D. K. Y.; Robinson, G.; Werth, J. Germination, persistence, and emergence of flaxleaf fleabane (*Conyza bonariensis* [L.] Cronquist). *Weed Biol. Manage.* **2007**, *7*, 192–199.

(11) Cruz-Hipolito, H.; Osuna, M. D.; Heredia, A.; Ruiz-Santaella, J. P.; De Prado, R. Nontarget mechanism involved in glyphosate tolerance found in *Canavalia ensiformis* plants. *J. Agric. Food Chem.* **2009**, *57* (11), 4844–4848.

(12) Weaver, S. E. The biology of Canadian weeds. 115. *Conyza canadensis*. *Can. J. Plant Sci.* **2001**, *81*, 867–875.

(13) Hao, J.-H.; Qiang, S.; Liu, Q.-Q.; Cao, F. Reproductive traits associated with invasiveness in *Conyza sumatrensis*. *J. Syst. Evol.* **2009**, *47* (3), 245–254.

(14) Duke, S. O.; Powles, S. B. Glyphosate: A once in a century herbicide. *Pest Manage. Sci.* **2008**, *64*, 319–325.

(15) Franz, J. E.; Mao, M. K.; Sikorski, J. A. *Glyphosate. A Unique Global Herbicide*; ACS Monograph 189; American Chemical Society: Washington, DC, 1997; 653 pp.

(16) Ferreira, E. A.; Galon, L.; Aspiazú, I.; Silva, A. A.; Concenco, G.; Silva, A. F.; Oliveira, J. A.; Vargas, L. Glyphosate translocation in hairy fleabane (*Conyza bonariensis*) biotypes. *Planta Daninha*. **2008**, *26*, 637–643.

(17) Steinrücken, H. C.; Amrhein, N. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. *Biochem. Biophys. Res. Commun.* **1980**, *94*, 1207–1212.

(18) Yu, Q.; Cairns, A.; Powles, S. B. Glyphosate, paraquat and ACCase multiple herbicide resistance evolved in a *Lolium rigidum* biotype. *Planta* **2007**, *225*, 499–513.

(19) Kaundun, S. S.; Zelaya, I. A.; Dale, R. P.; Lycett, A. J.; Carter, P.; Sharples, K. R.; McIndoe, E. Importance of the P106S target-site mutation in conferring resistance to glyphosate in a Goosegrass (*Eleusine indica*) population from the Philippines. *Weed Sci.* **2008**, *56*, 637–646.

(20) Harring, T.; Streibig, J. C.; Husted, S. Accumulation of shikimic acid: A technique for screening glyphosate efficacy. *J. Agric. Food Chem.* **1998**, *46*, 4406–4412.

(21) Hess, M.; Barralis, G.; Bleiholder, H.; Buhr, L.; Eggers, Th.; Hack, H.; Stauss, R. Use of the extended BBCH scale-general for the descriptions of the growth stages of mono and dicotyledonous weed species. *Weed Res.* **1997**, *37*, 433–441.

(22) Menendez, J.; Bastida, F.; De Prado, R. Resistance to chlortoluron in a downy brome (*Bromus tectorum*) biotype. *Weed Sci.* **2006**, *54*, 237–245.

(23) Ruiz-Santaella, J. P.; Heredia, A.; De Prado, R. Basis of selectivity of cyhalofop-butyl in *Oryza sativa* L. *Planta* **2006**, *223*, 191–199.

(24) Gauvrit, C. Glyphosate response to calcium, ethoxylated amine surfactant, and ammonium sulfate. *Weed Technol.* **2003**, *17*, 799–804.

(25) Grangeot, M.; Chauvel, B.; Gauvrit, C. Spray retention, foliar uptake and translocation of glufosinate and glyphosate in *Ambrosia artemisiifolia*. *Weed Res.* **2006**, *46*, 152–162.

(26) Perez-Jones, A.; Park, K. W.; Colquhoun, J.; Mallory-Smith, C.; Shaner, D. Identification of glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) in Oregon. *Weed Sci.* **2005**, *53*, 775–779.

(27) Koger, C. H.; Poston, D. H.; Hayes, R. M.; Montgomery, R. F. Glyphosate-resistant horseweed (*Conyza canadensis*) in Mississippi. *Weed Technol.* **2004**, *18*, 820–825.

(28) Shrestha, A.; Hembree, K. J.; Va, N. Growth stage influences level of resistance in glyphosate-resistant horseweed. *Calif. Agric.* **2007**, *61*, 67–70.

(29) De Ruiter, H.; Uffing, A. J. M.; Meinen, E.; Prins, A. Influence of surfactants and plant species on leaf retention of spray solutions. *Weed Sci.* **1990**, *38*, 567–572.

(30) Chachalis, D.; Reddy, K. N.; Elmore, C. D.; Steele, M. L. Herbicide efficacy, leaf structure, and spray droplet contact angle among

Ipomea species and smallflower morningglory. *Weed Sci.* **2001**, *49*, 628–634.

(31) Norsworthy, J. K.; Burgos, N. R.; Oliver, L. R. Differences in weed tolerance to glyphosate involved different mechanisms. *Weed Technol.* **2001**, *15*, 725–731.

(32) Michitte, P.; De Prado, R.; Espinoza, N.; Ruiz-Santaella, J. P.; Gauvrit, C. Mechanisms of resistance to glyphosate in a ryegrass (*Lolium multiflorum*) biotype from Chile. *Weed Sci.* **2007**, *55*, 435–440.

(33) Amrhein, N.; Deus, B.; Gehrke, P.; Steinrücken, H. C. The site of the inhibition of the shikimate pathway by glyphosate: II. Interference of glyphosate with chorismate formation *in vivo* and *in vitro*. *Plant Physiol.* **1980**, *66*, 830–834.

(34) Mueller, T. C.; Ellis, A. T.; Beeler, J. E.; Sharma, S. D.; Singh, M. Shikimate accumulation in nine weedy species following glyphosate application. *Weed Res.* **2008**, *48*, 455–460.